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Field-aged biochar stimulated N_2O production from greenhouse vegetable production soils by nitrification and denitrification☆

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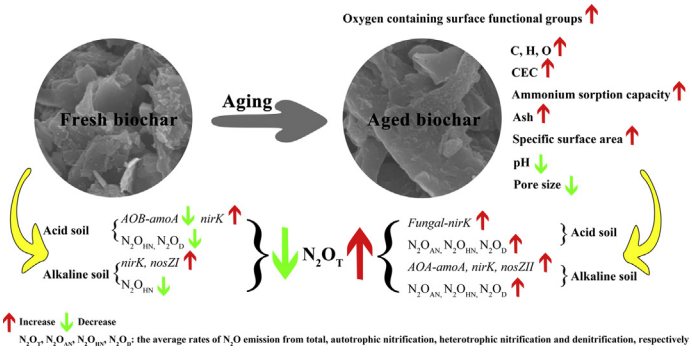
HIGHLIGHTS

- Aging can change the physical and chemical properties of biochar.
- Fresh biochar decreased N_2O *via* heterotrophic nitrification in vegetable soils.
- Aged biochar stimulated N_2O *via* nitrification & denitrification in vegetable soils.
- Aged biochar increased *Fungal-nirK* gene abundance in acid vegetable soil.

GRAPHICAL ABSTRACT



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ABSTRACT

Evidence suggests that biochar is among ideal strategies for climate change mitigation and sustainable agriculture. However, the effects of soil aging on the physicochemical characteristics of biochar and nitrous oxide (N_2O) production remain elusive. We set up a microcosm experiment with two greenhouse vegetable production (GVP) (alkaline and acid) soils by using the ^{15}N tracing technique and quantitative polymerase chain reaction (qPCR) to investigate the mechanisms of N_2O production as affected by fresh (FB) and aged biochar (AB) amendment. The results showed that AB increased the specific surface area, organic C, ammonium sorption capacity and cation exchange capacity, whereas decreased the pore size and pH relative to the FB. Results also demonstrated that FB effectively decreased N_2O emissions from both soils while it enhanced the abundance of *nirK* and *nosZI* genes in alkaline soil and reduced the abundance of ammonia-oxidizing bacteria (AOB) *amoA* and increased *nirK* and *nosZII* genes in acid soil. In contrast, AB significantly stimulated nitrification and denitrification in both soils and thus significantly increased the N_2O emissions by 43–78%. Furthermore, AB induced increases in ammonia-oxidizing archaeal (AOA) *amoA* and *nirK* gene abundances in alkaline soil and fungal *nirK* gene abundances in acid soil. These results suggest that AB may not be suitable for the mitigation of soil N_2O emissions in GVP soils thus improving our understanding of the potential mechanism of biochar in N_2O emissions.

1. Introduction

Globally, approximately 4% of the anthropogenic N in agricultural systems is returned to the atmosphere as nitrous oxide (N_2O) (Griffis et al., 2013) – a potent greenhouse gas and the predominant stratospheric ozone-depleting emission (Ravishankara et al., 2009) that is

☆ Main findings:Fresh biochar decreased N_2O mainly via heterotrophic nitrification, whereas aged biochar increased it through nitrification and denitrification in alkaline and acid vegetable soils.

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primarily produced by autotrophic nitrification, heterotrophic nitrification, and bacterial and fungal denitrification (Butterbach-bahl et al., 2013; Ma et al., 2017; Zhang et al., 2015). Greenhouse vegetable production (GVP) soils are the main sources of N_2O emissions in Chinese cropland due to increasing applications of both mineral nitrogen (N) fertilizers and manure (Wang et al., 2011); thus, strategies to reduce N_2O emissions in the context of global climate change mitigation are required.

Biochar is highly recalcitrant as a future climate change mitigation option (Weng et al., 2017; Woolf et al., 2016) and has been extensively used for improving soil structure, nutrient retention and as a method to sequester carbon (C) in agricultural soils (Hagemann et al., 2016). A biochar-induced reduction in N_2O emissions has been demonstrated in several lab and field experiments (Ameloot et al., 2007; Harter et al., 2014; Li et al., 2017; Liu et al., 2017). In contrast, some researchers have found that biochar does not impact (Angst et al., 2014; Case et al., 2017) or increase (Bamminger et al., 2017; Lin et al., 2017; Sánchez-García et al., 2014) soil N_2O emissions. On the one hand, the inconsistent effects of biochar on N_2O emissions depend on the interaction between biochar characteristics and soil properties (*e.g.*, soil pH, texture and soil water status) (Cayuela et al., 2014). On the other hand, changing characteristics of biochar with time in field and lab conditions has been observed. Though 3 year field-aged biochar effectively reduced N_2O emissions (Hagemann et al., 2017a), Felber et al. (2014) reported that biochar lost its initial ability to suppress soil N_2O emissions after 1 year field aging. The reapplication of biochar study also suggested the transient nature of biochar-mediated soil nutrient dynamics and microbial growth (Quilliam et al., 2012). The differences in the response of biochar amendments on N_2O emissions might be linked to microbial mediated processes of N_2O production. N_2O reduction is attributed to the selection of the denitrification pathway in acid and alkaline soils (through increased *nosZI- or nosZII*-encoded bacterial N_2O reductase) (Harter et al., 2014; Krause et al., 2018; Xu et al., 2014), while increased N_2O emissions have been attributed to the selection of the nitrification pathway in acid soil (through increased soil pH, which in turn increased bacterial *amoA*) (Lin et al., 2017).

It's evident that the aging of biochar affected soil N dynamics (Mia et al., 2017c) and shaped N_2O-genic microbial activity (*e.g.*, nitrifying and denitrifying) (Harter et al., 2017; Krause et al., 2018) with changing physicochemical properties (*e.g.*, pH, sorption capacity, toxicity, surface area) and forming a range of biochar-derived organic materials (De et al., 2018). Moreover, soil acidification, salinization, and nutrient imbalance can be induced due to the high cropping index, large fertilizer input, and closed environment (Hu et al., 2017), which further affecting N transformation processes (Zhu et al., 2011). To our knowledge, there are only two studies that investigated the impact of aged biochar (AB) on the abundance of functional genes in N_2O-forming and reducing microorganisms (Hagemann et al., 2017a) and gross N-transformation rates (Mia et al., 2017c). The quantitative polymerase chain reaction (qPCR) suggests that the 3 year field-aged biochar did not change the abundance of N_2O-related functional genes, but was able to suppress N_2O emissions (Hagemann et al., 2017a). Since soil N_2O emissions and N-transformation pathways are controlled by soil properties (Liu et al., 2018), fresh biochar (FB) and aged biochar (AB) may interact with different soil properties to influence the N-transformation rates and microbial communities, and thus affecting the related N_2O production.

Despite the fact that biochar-induced reduction in N_2O emissions has been reported in GVP soils (Fan et al., 2017; Li et al., 2017), little attention has been paid to examine the biochar's persistence of the N_2O emission-suppressing effect. In the present study, we designed a microcosm experiment supplied with FB and AB under alkaline and acid GVP soils to determine the processes (autotrophic nitrification, heterotrophic nitrification and denitrification) involved in N_2O production. The objective of this study was to evaluate (1) whether field soil aging could influence biochar properties involving composition, physical and chemical characteristics and (2) if so, how the rates of gross N transformation and the abundance of functional genes of N_2O-forming and N_2O-reducing microorganisms (*e.g.*, *bacterial* and *archaeal amoA*, *denitrifiers nirS*, *nirK* and *nosZI*, *nosZII* and *fungal nirK gene*) are related to N_2O production pathways in biochar-amended GVP soils. We hypothesize that the responses of biological N_2O production to biochar amendment vary with biochar aging and differ between the alkaline and acid GVP soils.

2. Materials and methods

2.1. Biochar preparation

The field-aged biochar particles were separated from a field biochar-based experiment that has been previously described (Xu et al., 2016) (details in Supporting Information). In July 2017 (~5 years after biochar application), random soil-biochar mixture samples were collected from a 0–15 cm depth interval from these field strips following harvest.

The procedure of separating aged biochar from the soil was according to Dong et al. (2017). Furthermore, the fresh biochar (FB) and aged biochar (AB) were ground to a particle size <1 mm and stored at 4 °C until being analyzed. Further details concerning the separate description and biochar properties analyses can be found in Supplementary Information (Fig. S1).

2.2. Microcosm incubation

Two GVP soils were selected for the experiments with a long vegetable cropping history (>15 years) of conventional cultivation (Table S1). A Fimi-Orthic Anthrosol in NanJing (32°01′ N, 118°52′ E) and a Ferralic Cambisol in JianNing (26°45′ N, 116°32′ E) (FAO and ISRIC, 2012) were named as the alkaline soil and the acid soil, respectively, based on their soil pH. Samples were taken from the upper 20 cm, air-dried, sieved to 2 mm and stored at 4 °C until incubation experiments were performed. One week before the start of the incubation, the soil was pre-incubated with 55–60% water-filled pore space (WFPS) and at 25 °C to remove inherent labile C constituents. Then, the sieved biochars were thoroughly mixed with the soils at a rate of 1% dry weight soil (equivalent to 20 t ha^{-1}) to obtain a completely homogeneous mixture.

The laboratory ^{15}N labelling experiment was conducted in 120-ml serum vials containing 20 g of soil (oven-dry weight basis) to explore the effects of biochar on the processes involved in N_2O production. There were twelve different treatments, and treatments for each soil are as follows: (1) $^{15}NH_4NO_3$ + CK (no biochar), (2) $^{15}NH_4NO_3$ + FB, (3) $^{15}NH_4NO_3$ + AB, (4) $NH_4^{15}NO_3$ + CK, (5) $NH_4^{15}NO_3$ + FB and (6) $NH_4^{15}NO_3$ + AB.

It is worth noting that the higher NO_3^- contents in acid soil (Table S1) require greater ^{15}N enrichment of $NH_4^{15}NO_3$ to provide sufficient measurement precision. After pre-incubation, 2 ml of $^{15}NH_4NO_3$ (10.28 atom% ^{15}N excess for both soils) or $NH_4^{15}NO_3$ (10.28 and 20 atom% excess for the alkaline and acid soils, respectively) solution was added to each serum vial at a rate of 150 mg N kg^{-1} soil (75 mg NH_4^+-N kg^{-1} soil and 75 mg NO_3^--N kg^{-1} soil). The microcosms were incubated in the dark for 21 days at 25 °C after adjusting the soil to 65% WFPS. Aerobic conditions and soil moisture contents in the vials were maintained every 3 days by opening the microcosms for aeration and water replenishment.

Headspace gas samples were collected at 1, 2, 5, 7, 11, 15, and 21-day after fertilizer application. Prior to gas sampling, the vials were opened for 30 min to renew the atmosphere inside and resealed for 6 h. A total of ~35 ml of gas was collected with 50-ml plastic syringe from each vial and injected into serum vials for the $^{15}N_2O$ analysis (22 ml) and the surplus gas in the syringe for N_2O concentration analysis. A destructive sampling method was used, with triplicate vials per soil type and biochar amendment sacrificed at 1, 7 and 21 days for $^{15}NH_4^+$-N, $^{15}NO_3^-$-N (including concentrations), insoluble organic ^{15}N and $^{15}N_2O$

analysis. Gas, soil sampling and analysis of concentrations and isotopic signatures were summarized briefly in Supplementary Methods.

2.3. Soil DNA extraction and quantitative PCR (qPCR)

The soil samples were collected from the incubation containers after 21 days of incubation and used for DNA extraction and quantitative PCR analysis (qPCR). The ammonia-oxidizing bacterial (AOB) and archaea (AOA) *amoA* and the denitrifying bacterial *nirK*, *nirS* and fungal *nirK* genes were used to target the N_2O-producers (Bru et al., 2011; Long et al., 2015), while the *nosZI* and *nosZII* genes were used to target the N_2O-reducers (Jones et al., 2013). The functional genes of the ammonia oxidizers (AOA and AOB *amoA*) and denitrifiers (*nirK*/*S*, *nosZI*) were assessed using the primers described in Harter et al. (2014). The abundance of *nosZII* and the fungal *nirK* gene were assessed using the primers described in Jones et al. (2013) and Long et al. (2015), respectively (further details on soil DNA extraction and gene-specific primers are given in Table S2 in the Supporting Information).

2.4. Determining the N-transformation rates and contribution of different processes to N_2O production

The gross N mineralization and nitrification rates were calculated by the ^{15}N isotopic pool dilution method according to Davidson et al. (1991). The dissimilatory nitrate reduction to ammonium and autotrophic nitrification (AN) rates were calculated based on the equations of Huygens et al. (2008) with data from the $^{15}NO_3^-$ and $^{15}NH_4^+$ labeling treatments, respectively (detailed equations to calculate N transformation rate are given in the Supporting Information). Heterotrophic nitrification (HN) was calculated as the difference between the gross nitrification rate and autotrophic nitrification (Barraclough and Puri, 1995). The immobilization rates of NH_4^+ or NO_3^- and remineralization of N were calculated by the method described by Shindo and Nishio (2005).

Here, we assumed that N_2O originates from NH_4^+, organic N and NO_3^- *via* autotrophic nitrification, heterotrophic nitrification and denitrification, respectively, the ^{15}N atom fraction of the resulting N_2O mixture (αN_2O) can be determined using the following equation (Rutting et al., 2010):

$$\alpha N_2O = C_{AN} \times \alpha_n + C_{AH} \times \alpha_h + C_D \times \alpha_d \quad (1)$$

where C_{AN}, C_{AH}, and C_D are the fractions related to the NH_4^+, organic N and NO_3^- pools, respectively, and α_n, α_h, and α_d, represent the ^{15}N abundance of the NH_4^+, organic N and NO_3^-, respectively.

Rates of N_2O production from autotrophic nitrification (N_2O_{AN}), heterotrophic nitrification (N_2O_{HN}), and denitrification (N_2O_D) were calculated as follows:

$$N_2O_{AN} \text{ or } N_2O_{AH} \text{ or } N_2O_D = C_{AN} \text{ or } C_{AH} \text{ or } C_D \times N_2O_T \quad (2)$$

where N_2O_T is the total N_2O emission rate from the tested soil.

The ratio of N_2O emissions from autotrophic nitrification (R_{AN}) and heterotrophic nitrification (R_{HN}) to the corresponding gross rates were calculated as follows:

$$R_{AN} \text{ or } R_{HN} = \frac{N_2O_{AN}}{AN} \text{ or } N_2O_{HN}/HN \quad (3)$$

2.5. Data processing and statistical analyses

The ^{15}N abundance (atom fraction ^{15}N) of soil-emitted N_2O ($A(^{15}N)_{em}$) was calculated from that of the headspace samples, $A(^{15}N)_{mix}$ and the ambient air, $A(^{15}N)_{air}$, assuming two-component mixing:

$$A(^{15}N)_{mix} \times C_{mix} = A(^{15}N)_{em} \times C_{em} + A(^{15}N)_{air} \times C_{air} \quad (4)$$

where C is the N_2O concentration and $C_{mix} = C_{em} + C_{air}$. Emissions corresponding to cem < 10 nl L^{-1} had to be disregarded due to large relative errors when calculating $A(^{15}N)_{em}$ (Toyoda et al., 2011) and in these cases, $A(^{15}N)_{mix}$ was set to $A(^{15}N)_{air}$.

The different properties between fresh and aged biochar as well as alkaline and acid soils were analyzed by *t*-tests ($p < 0.05$). Repeated-measures analyses of variance (ANOVA) were conducted to test the significance of soil type, sampling time, treatment and the associated interactions on N_2O emissions, inorganic N concentration and gross N transformation rate. A two-way ANOVA was used to analyze the effects of soil type, treatment and the associated interactions on the contribution percentages of autotrophic nitrification, heterotrophic nitrification and denitrification to N_2O emissions, the rate of these processes, and the ratio of N_2O emission from autotrophic nitrification and heterotrophic nitrification. A one-way ANOVA and an LSD test were used to determine significant differences ($p < 0.05$). All data were analyzed using the SPSS 20.0 (IBM Co., Armonk, NY, USA) and Origin Pro 8.1 (OriginLab, Northampton, MA, USA).

3. Results

3.1. Biochar properties

Higher levels of SSA (specific surface area), OC (organic carbon), H (hydrogen), O (oxygen), pore volume, ash content, CEC (cation exchange capacity) and μ (maximum NH_4^+-N sorption capacity) were observed in AB than those in FB (Fig. 1a; Table S1; Fig. S2). However, FB had higher levels of pH and pore size than AB (Table S1). The ^{13}C NMR spectra of FB and AB are similar and dominated by an intense and broad signal at 127 ppm corresponding to aromatics (Fig. 1b). Both FB and AB showed a stretching signal at 874 cm^{-1} for out-of-plane bending of the aromatic C—H bonds, and at 1420 and 1560 cm^{-1} for vibrations of COO— (Fig. 1c). Compared with FB, the bands at 1420 cm^{-1} and 1560 cm^{-1} assigned to COO— increased and the stretching vibration combination of C—O and O—H deformation at 1030 cm^{-1} appeared in AB (Fig. 1c).

3.2. Nitrifier (AOA-amoA and AOB-amoA), denitrifier (nirK, nirS, nosZI and nosZII) and fungal-nirK functional gene abundances

The abundance of the *AOA-amoA* gene in alkaline soil was significantly increased in the AB treatment ($p < 0.01$, Fig. 2). The abundance of the *AOB-amoA* gene in acid soil was significantly higher under the CK ($p < 0.01$) and AB ($p < 0.05$) treatments than the FB treatment. No difference in abundance of the *AOA*- and *AOB-amoA* gene was found among CK, FB and AB in both soils ($p > 0.05$).

The abundance of the *nirK* gene in alkaline soil was significantly higher under the FB and AB treatments ($p < 0.01$) than the CK treatment, and it was significantly increased by the FB treatment ($p < 0.05$) in acid soil. No difference in the abundance of *nirS* genes was found among CK, FB and AB in both types of soil ($p > 0.05$, Fig. 2).

The abundance of *nosZI* genes in alkaline soil was significantly higher under the FB ($p < 0.01$) treatment than the CK and AB treatments, but no difference in the abundance of the *nosZI* gene was found among CK, FB and AB in acid soil ($p > 0.05$). Compared with the CK and AB treatments, the abundance of *nosZII* in the FB treatment was significantly increased ($p < 0.01$) in acid soil, while there was no significant difference among CK, FB and AB in alkaline soil ($p > 0.05$, Fig. 2).

There was no significant difference in abundance of the *fungal-nirK* gene among CK, FB and AB in alkaline soil ($p > 0.05$). The AB treatment significantly increased *fungal-nirK* gene abundance in acid soil, but no difference in abundance of the *fungal-nirK* gene was found between the CK and FB treatments ($p > 0.05$, Fig. 2).

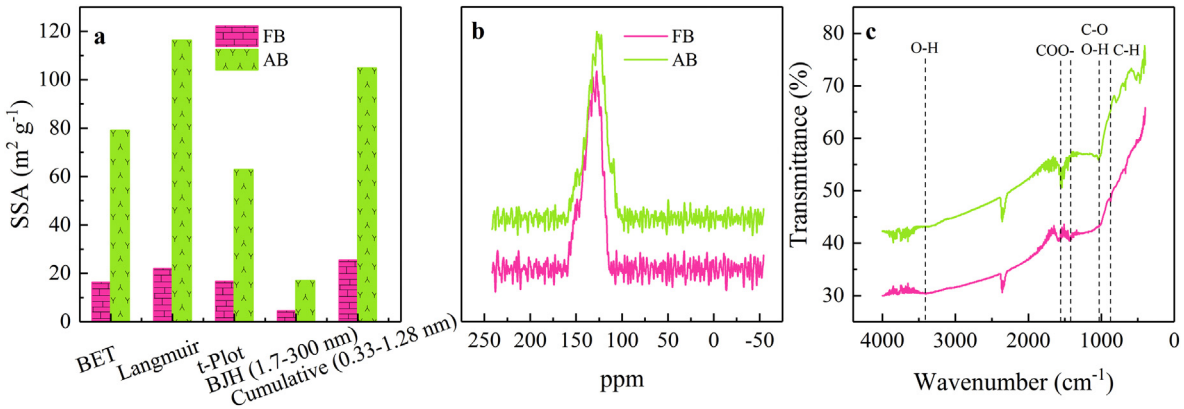

Fig. 1. The spectra and specific surface area (SSA) (a), solid-state ^{13}C NMR (b), FTIR (c) for fresh and aged biochar. The SSA according to N_2 gas adsorption. Total SSA according to the Brunauer, Emmett and Teller method (BET); SSA for pores ranging from 1.7 nm to 300 nm according to the Barrett, Joyner and Halenda method (BJH).

3.3. Contributions of nitrification and denitrification to N_2O emission

The average total N_2O emission rate (N_2O_T) was significantly reduced by 10% and 14% by FB amendment but was increased by 44% and 75% by AB amendment in the alkaline and acid soils, respectively (Table 1).

In the $^{15}NH_4^+$- and $^{15}NO_3^-$-labeled treatments, the ^{15}N enrichment in the N_2O pool was always between the ^{15}N enrichment levels of the NH_4^+ and NO_3^- pools, suggesting that N_2O was produced by both nitrification and denitrification (Fig. S4). The AN was the main source of N_2O in alkaline soil in all the treatments, comprising between 59% and 65% of the cumulative N_2O flux (Fig. 3b, Table 1). The cumulative N_2O fluxes from AN, HN, and D were the highest in the AB treatment (Fig. 3b). N_2O emission rates from autotrophic nitrification (N_2O_{AN}) and denitrification (N_2O_D) in the alkaline soil of the AB treatment were significantly higher than the emissions from the CK treatment ($p < 0.05$, Table 1). There were no significant differences in the N_2O product ratio of autotrophic nitrification (R_{AN}) and heterotrophic nitrification (R_{HN}) among the CK, FB and AB treatments in the alkaline soil ($p > 0.05$), but R_{HN} was higher than R_{AN} for the AB treatment ($p < 0.01$, Table 1).

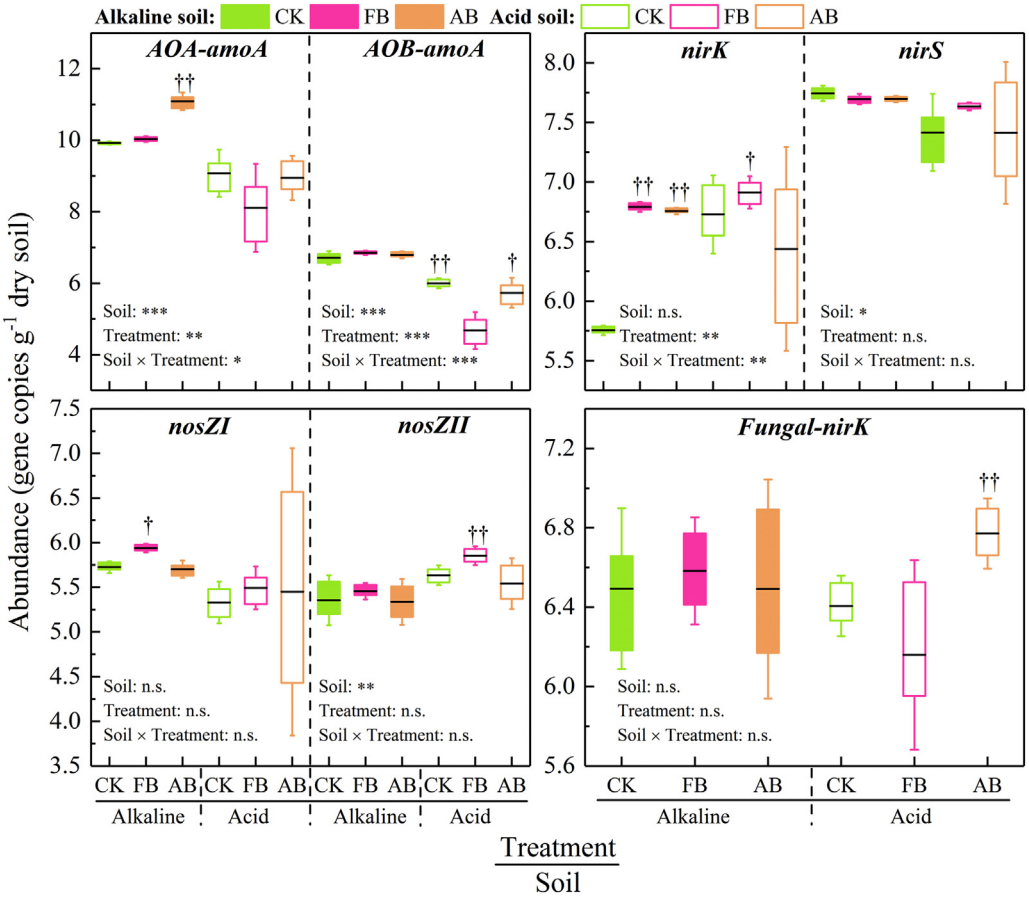

Fig. 2. The log-10-transformed gene abundances per g dry soil of the targeted functional guilds (*AOA-amoA*, *AOB-amoA*, *nirS*, *nirK*, *nosZI*, *nosZII* and *Fungal-nirK*) in alkaline and acid soils without (CK) or with fresh (FB) or aged (AB) biochar applications after a 21-day incubation. Error bars indicate standard deviations. The results of a two-way ANOVA on gene abundances for the two factors (soil type, and treatments) and their interactions are also shown in the figure. **, * and n.s. indicate significance at $p < 0.01$, $p < 0.05$, and no significance, respectively. †† and † indicate significantly higher gene abundances in a specific treatment than others for each soil at $p < 0.01$ and $p < 0.05$, respectively.

Table 1
Average contribution percentages of autotrophic nitrification, heterotrophic nitrification and denitrification to N_2O emissions and the rate from these processes, and the ratio of N_2O emissions from autotrophic nitrification and heterotrophic nitrification relative to the respective gross rates over 21 days (mean ± standard error, $n = 3$).

Soil type	Treatment	Contribution			N_2O emission rate ($\mu g\ N\ kg^{-1}\ d^{-1}$)				Ratio (‰)	
		C_{AN}[a]	C_{HN}	C_D	N_2O_T	N_2O_{AN}	N_2O_{HN}	N_2O_D	R_{AN}	R_{HN}
Alkaline	CK[b]	0.65 ± 0.05(a)[c]	0.12 ± 0.05(a)	0.23 ± 0.02(b)	1.56 ± 0.05(b)	1.02 ± 0.03(b)	0.19 ± 0.05(b)	0.36 ± 0.01(b)	0.20 ± 0.01(a)	0.16 ± 0.16(a)
	FB	0.60 ± 0.12(a)	0.09 ± 0.12(a)	0.31 ± 0.04(a)	1.40 ± 0.04(c)	0.98 ± 0.03(b)	0.14 ± 0.02(c)	0.36 ± 0.02(b)	0.21 ± 0.02(a)	0.20 ± 0.04(a)
	AB	0.59 ± 0.11(a)	0.15 ± 0.08(a)	0.26 ± 0.01(a)	2.25 ± 0.05(a)	1.33 ± 0.03(a)	0.34 ± 0.01(a)	0.59 ± 0.04(a)	0.24 ± 0.03(a)	0.81 ± 0.86(a)
Acid	CK	0.18 ± 0.09(a)	0.41 ± 0.05(a)	0.41 ± 0.10(a)	1.01 ± 0.05(b)	0.18 ± 0.03(b)	0.41 ± 0.02(b)	0.41 ± 0.02(b)	0.02 ± 0.01(a)	0.54 ± 0.11(a)
	FB	0.13 ± 0.06(a)	0.43 ± 0.04(a)	0.44 ± 0.10(a)	0.86 ± 0.06(c)	0.19 ± 0.01(b)	0.29 ± 0.03(c)	0.31 ± 0.02(c)	0.02 ± 0.01(a)	0.27 ± 0.05(b)
	AB	0.18 ± 0.13(a)	0.39 ± 0.08(a)	0.43 ± 0.12(a)	1.75 ± 0.06(a)	0.31 ± 0.05(a)	0.68 ± 0.04(a)	0.75 ± 0.03(a)	0.03 ± 0.01(a)	0.44 ± 0.05(a)
Results of two-way ANOVA testing the effects of soil type, treatment, and their interactions on C_{AN}, C_{HN}, C_D, N_2O_T, N_2O_{AN}, N_2O_{HN}, N_2O_D, R_{AN}, and R_{HN}										
Soil		**[d]	***	**	**	**	n.s.	*	**	*
Treatment		***	n.s.	*	***	**	***	***	n.s.	*
Soil × Treatment		**	***	**	*	n.s.	**	**	n.s.	*

[a] CAN, CHN, CD: the contribution percentage of N2O emissions from autotrophic nitrification, heterotrophic nitrification and denitrification, respectively; N2OT, N2OAN, N2OHN, N2OD: the average rates of N2O emissions from total, autotrophic nitrification, heterotrophic nitrification and denitrification, respectively; RAN, RHN: the ratios of N2O emissions from autotrophic nitrification and heterotrophic nitrification, relative to their gross rates, respectively.
[b] CK: no biochar; FB: fresh biochar; AB: aged biochar.
[c] Different lowercase letters within a column denote significant differences at $p < 0.05$ for each soil.
[d] Based on a two-way ANOVA. n.s., not significant, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

For the acid soil, the average contribution of HN to N_2O emissions (C_{HN}) ranged from 39% in AB treatment to 44% in the FB treatment, which was comparable to denitrification (41%–44%) (Table 1). The cumulative N_2O fluxes from AN, HN, and D were the highest in the AB treatment and lowest in the FB treatment (Fig. 3b). N_2O emission rates from N_2O_{AN}, N_2O_{HN} and N_2O_D in the acid soil of the AB treatment were significantly higher than the emissions from the CK treatment ($p < 0.05$), and N_2O emission rates from N_2O_{HN} and N_2O_D were significantly reduced in the FB treatment ($p < 0.05$, Table 1). The R_{HN} was significantly higher in the CK and AB treatments than that in the FB treatment under acid soil ($p < 0.05$, Table 1).

4. Discussion

4.1. Variation in biochar properties after field-aged

Artificial aging, mild chemical oxidation or field aging of biochar has been shown to increase SSA (Dong et al., 2017; Hagemann et al., 2017b; Mia et al., 2017a). In this study, SSA increased by 200–500% (Fig. 1a), and the total pore volume increased by 267%, while the pore size decreased by 28% of biochar with aging after 5 years in the field (Table S1), which suggested that new pores formed during the field aging process (Dong et al., 2017). This outcome is contrary to that of Martin et al. (2012) who found the SSA of aged biochar decreased by blocking or filling out pore space with soil organic matter.

In line with the finding that the ^{13}C NMR spectra exhibited aromatic peaks in the biochar with 5 years field aging (Dong et al. (2017), we found that the ^{13}C NMR spectra of FB and AB are dominated by an intense and broad signal at 127 ppm corresponding to aromatic C (Fig. 1b). The aromatic properties of biochar did not change significantly in GVP soils in our study.

Similar to others studies, the intensity and relative peak area for the carboxylic group (—COOH) band at ~1560 cm^{-1} increased in the aged, relative to fresh biochar, suggesting that age caused carboxylation (Dong et al., 2017; Mia et al., 2017a). Compared with FB, AB also caused functionalization, showing absorption bands at ~1030 cm^{-1} for C—O and O—H (Fig. 1c), which suggested that the interaction of soil promoted the formation of C—O. These results indicated that the increase

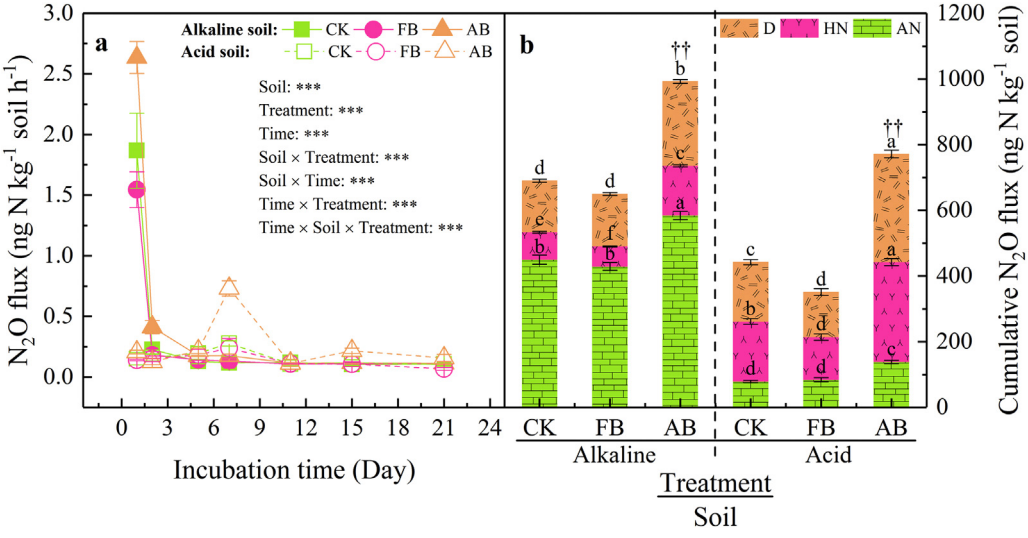

Fig. 3. N_2O flux (a) and cumulative N_2O flux *via* three processes (b) (autotrophic nitrification-AN, heterotrophic nitrification-HN, denitrification-D) in alkaline and acid soils without (CK) or with the application of fresh (FB) or aged (AB) biochar between 1 day and 21 days after labeling. The cumulative flux per process is an average over the three plots per treatment. Error bars indicate standard deviations. The results of a three-way repeated measures ANOVA on the N_2O flux for the three factors (soil type, sampling time and treatments) and their interactions are also shown in the figure. **, * and n.s. indicate significance at $p < 0.01$, $p < 0.05$, and no significance, respectively. Different letters denote significant differences among treatments for each process ($p < 0.05$). †† and † indicates significantly higher cumulative fluxes in the AB treatment compared to CK and FB treatments for each soil at $p < 0.01$, $p < 0.05$, respectively.

in O content was not merely due to the sorption of O in the aged biochar surfaces but was due to the formation of O-containing functional groups during the aging process (Cheng et al., 2008). It was shown that the presence of oxidized functional groups on the surface of biochar improved soil fertility by increasing the CEC (Liu et al., 2013), which can be proved by the results of O and C biochar and the CEC (Table S1); thus, a greater nutrient retention is expected in AB (*e.g.*, μ value, IA and IN). These results are consistent with those findings during the chemical oxidation (Mia et al., 2017a) and field aging processes (Hagemann et al., 2017b).

We also found that the OC content of AB was 23% higher than that of FB (Table S1). This suggests that the OC may be absorbed to the aged biochar surfaces. Hagemann et al. (2017b) demonstrated that biochar aging facilitated interaction with soil minerals and the formation of an organic coating in soil-aged biochar.

4.2. N_2O emissions as affected by FB and AB

Independent of biochar addition, autotrophic nitrification was found to be the main source of N_2O in alkaline soil, whereas heterotrophic nitrification and denitrification were the main N_2O-producing pathway in acid soil (Table 1, Fig. 3b). This finding is in agreement with other studies (Case et al., 2017; Harter et al., 2016). However, FB and AB significantly affected the N_2O emission rate from nitrification or denitrification in both soils (Table 1). Biochar may influence different N_2O-producing processes by altering soil aeration, water retention, N-availability, and soil pH or by sorption of labile C, leading to shifts in the nitrifying and denitrifying soil microbial community (Cayuela et al., 2014; Clough et al., 2013).

In the current study, the observed N_2O emissions were effectively decreased by FB addition in both types of soil (Table 1, Fig. 3). In alkaline soil, we found that FB had a significant effect on N_2O_T but not N_2O_{AN} or N_2O_D (Table 1). Therefore, the primary reason that FB affects N_2O emissions appears to be its inhibitory effect on heterotrophic nitrification. Furthermore, chemically mediated effect of biochar (soil pH increase) acted as the driving factor in improving the activity of N_2O-producing and -reducing organisms (Wang et al., 2013; Liu et al., 2017). The FB addition significantly increased abundance of *nirK*-nitrite-reducing bacteria ($p < 0.05$), and *nosZI*-N_2O-reducing bacteria in alkaline ($p < 0.05$) and *nosZII*-N_2O-reducing bacteria in acid soil ($p < 0.01$, Fig. 2). The FB addition might not only enhance the steps of NO_3^- reduction towards N_2O, but also lead to a stronger further reduction of N_2O to N_2, culminating in a net decline of soil N_2O emissions; N_2O consumption even outweighed production with FB amendment in acid soil (Table 1, Fig. 3). Recent studies revealed that approximately half of the atypical *nosZ* genes (*nosZII*) carrying N_2O reducers detected so far lack other functional denitrification genes and thus depend on the supply of N_2O by other organisms (Jones et al., 2014; Orellana et al., 2014). Harter et al. (2016) reported a significant increase in *nosZII* transcript copies, indicating a biochar-induced promotion of N_2O-reducing microorganisms. In our case, the biochar's liming effect was more likely to favor the growth of *nosZII*-reducing microorganisms in acid GVP soil, where denitrification served as the main N_2O production pathway. The observed increase in gross nitrification along with a decrease in immobilization of nitrate may indirectly support the possible accelerated N transformations in FB-amended acid soil with a facilitation of substrate consumption to denitrifiers (Tables 1 and S3). Notably, FB had a negative influence on the R_{HN} in the present study (Table 2), which has seldomly been reported. Thus, the FB produced triple functions, *i.e.*, inhibiting the heterotrophic nitrification and denitrification rates and reducing the fraction of N_2O in the nitrification products in the tested acid GVP soil. Certain compounds in biochar are known as microbial inhibitors to inhibit nitrification or denitrification, such as ethylene (Spokas et al., 2011). Thus, we cannot exclude the possibility that the potential toxicity of FB might have affected the growth and activity of nitrifiers.

A negative effect (Hagemann et al., 2017a), positive effect (Spokas, 2013) or lack of an effect (Bamminger et al., 2017) of field-aged biochar on N_2O mitigation from microcosms and field studies have been reported. In our study, AB effectively increased N_2O emissions by ~43% in alkaline and ~78% in acid soil (Fig. 3b). It is remarkable that the stimulation of N_2O emissions of AB was observed to be pronounced in the microcosm after biochar aging for 5 years. The N_2O_T, N_2O_{AN}, N_2O_{HN} and N_2O_D were significantly increased with AB addition in both types of soil ($p < 0.05$, Table 1). Therefore, our findings suggest that the enhanced rates of N_2O emission from nitrification and denitrification induced by AB may be the main reason for N_2O emission stimulation. Besides, the AB treatment exhibited greater N mineralization rates compared to FB, which was affected by the changing density of hydrophilic functional groups in AB (Fig. 1), consistent with the observed oxygenated surface (Mia et al., 2017a; Wang et al., 2017). In addition, greater carboxyl and hydroxyl functional group density could allow for greater nutrient retention at the surface and potentially promote greater microbial activity (Glaser et al., 2001). This is supported in part by the higher OC content of AB than FB (Table S1). Assuming that the NH_4^+ and NO_3^- provided by mineralized organic N was rapidly nitrified and denitrified, we suggest that AB amendment did increase soil NH_4^+ and NO_3^- availability.

The significant differences in the abundance of the nitrifying and denitrifying microbial community between alkaline and acid soils were observed in response to soil biochar amendments (Fig. 2). Alkalinity induced by biochar increases nitrification, which occurs rapidly at pH > 6 (Nguyen et al., 2017). Similarly, we found that the AOA-*amoA* gene abundance and accompanied by GN rate were significantly increased in response to the addition of biochar in the alkaline soil ($p < 0.05$, Fig. 2, Table S3). This is in agreement with Harter et al. (2014), who reported the AOA-*amoA* gene abundance was increased in biochar-amended alkaline soil after 29-day of incubation. A previous study showed that some *nirS*- and *nirK*-containing denitrifiers lack the genetic capacity to reduce N_2O and are suggested to be major contributors to N_2O production during denitrification (Philippot et al., 2011). Thus, an increase in the amount of N_2O-producing bacteria (containing the *nirK* gene) and archaea by AB might be the explanation for the increase in N_2O emissions in the alkaline soil. Meanwhile, most fungi lack N_2O reductase, resulting in N_2O as the final denitrification product (Saggar et al., 2013). Previous studies demonstrated that organic matter could increase the relative contribution of fungi to N_2O emissions in acid soils (Ma et al., 2017; Xu et al., 2017). A possible explanation for this increase in N_2O emissions *via* denitrification in acid soil is as a result of higher OC content of aged biochar (Table S1). Moreover, AB amendment stimulated gross heterotrophic nitrification by labile, biodegradable C (Hagemann et al., 2017a), which also explained for the increase in N_2O. Compared with the CK, the N_2O_{HN} was stimulated by biochar addition, especially for the AB treatment in the acid soil (Table 1). Biochar-treated soils exhibited nitrification rates 3–10 times higher than mineralization rates (Table 1). This dichotomy between NH_4^+ supply and nitrification rates could point to the increased importance of heterotrophic nitrification fueled by organic N compounds in the AB treatment (Table 1), which is consistent with report by Prommer et al. (2014).

The current understanding of the change in surface properties of biochar due to aging in soils is shown in Fig. S5 illustrating the potential mechanisms that biochar may influence N_2O production. Our results, increased N_2O emissions in the aged biochar treatment, may be explained by: (i) an increased CEC or adsorption, which maintained and enhanced N-cycling rates (Mia et al., 2017b); (ii) stimulated SOM mineralization, providing more available C or N and creating anaerobic zones or hot spots for denitrification (Verhoeven and Six, 2014); and (iii) the formation of a large SSA, which is a habitat for microbes (Ng et al., 2014), potentially promoting nitrification (Prommer et al., 2014). Another mechanism resulting in increased N_2O emissions could be due to the loss (*i.e.*, degradation or desorption) of biochar-sorbed nitrification/denitrification inhibitors through field aging, which was proved by Spokas

(2013) that aged biochar did possess a significantly lower quantity of the original absorbed organic compounds (*e.g.*, furans, furfurals, and alcohols) than fresh biochar.

It's worth noting that the effects of biochar on the nucleic acid extraction efficiency must be exercised when comparing microbial abundance and diversity with different biochar additions. Dai et al. (2017) found that the DNA extraction efficiency from soil decreased even in the presence of high-ash biochar, with unexpected changes over time. This illustrated that, specifically in the absence of aged biochar, the nucleic acid extraction efficiency can be low, with increasing aromaticity and surface area due to hydrophobic adsorption of DNA in our study. Future research employing the characterization of nucleic acids extracted from biochar-amended soils should consider how changes in extraction efficiency may affect data interpretation.

5. Conclusions

Biochar was aged in an experimental agriculture field for 5 years, which increased the CEC, SSA, and pore volume but decreased the pore size and pH, and also changed the elemental composition. Results show that the application of aged biochar to alkaline and acid soils accelerated N bio-availability through increased gross N mineralization, immobilization and nitrification rates. Altogether, the N_2O emissions were significantly decreased by fresh biochar due to its negative effect on heterotrophic nitrification in alkaline soil and both heterotrophic nitrification and denitrification in acid soil. The fresh biochar amendment also increased the abundance of *nosZI* and *nosZII* in both soils. Interestingly, the application of aged biochar significantly increased the N_2O produced from nitrification and denitrification in both soils, accompanied by a significant increase in the abundance of *AOA-amoA* and *nirK* in alkaline soil and *Fungal-nirK* in acid soil. Clearly, the linkages between biochar properties, N transformation rates, soil microbial communities, and N_2O emissions in agroecosystems need to be further explored in long-term field experiments to evaluate biochar as a C sequestration and N_2O mitigation tool.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.06.166.

References

Ameloot, N., Maenhout, P., Neve, S.D., Sleutel, S., 2007. Biochar-induced N_2O emission reductions after field incorporation in a loam soil. Geoderma 267, 10–16.

Angst, T.E., Six, J., Reay, D.S., Sohi, S.P., 2014. Impact of pine chip biochar on trace greenhouse gas emissions and soil nutrient dynamics in an annual ryegrass system in California. Agric. Ecosyst. Environ. 191, 17–26.

Bamminger, C., Poll, C., Marhan, S., 2017. Offsetting global warming-induced elevated greenhouse gas emissions from an arable soil by biochar application. Glob. Chang. Biol. 24, e318–e334.

Barraclough, D., Puri, G., 1995. The use of ^{15}N pool dilution and enrichment to separate the heterotrophic and autotrophic pathways of nitrification. Soil Biol. Biochem. 27, 17–22.

Bru, D., Ramette, A., Saby, N.P.A., Dequiedt, S., Ranjard, L., Jolivet, C., et al., 2011. Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. ISME J. 5, 532–542.

Butterbach-bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R., Zechmeisterboltenstern, S., 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philos. Trans. R. Soc. B 368, 91–97.

Case, S.D.C., Uno, H., Nakajima, Y., Jensen, L.S., Akiyama, H., 2017. Bamboo biochar does not affect paddy soil N_2O emissions or source following slurry or mineral fertilizer amendment-a ^{15}N tracer study. J. Plant Nutr. Soil Sci. 181, 90–98.

Cayuela, M.L., Zwieten, L.V., Singh, B.P., Jeffery, S., Roig, A., Sánchez-Monedero, M., 2014. Biochar's role in mitigating soil nitrous oxide emissions: a review and meta-analysis. Agric. Ecosyst. Environ. 191, 5–16.

Cheng, C.H., Lehmann, J., Engelhard, M.H., 2008. Natural oxidation of black carbon in soils: changes in molecular form and surface charge along a climosequence. Geochim. Cosmochim. Acta 72, 1598–1610.

Clough, T.J., Condron, L.M., Kammann, C., Müller, C., 2013. A review of biochar and soil nitrogen dynamics. Agronomy 3, 275–293.

Dai, Z.M., Webster, T.M., Enders, A., Hanley, K.L., Xu, J.M., Thies, J.E., et al., 2017. DNA extraction efficiency from soil as affected by pyrolysis temperature and extractable organic carbon of high-ash biochar. Soil Biol. Biochem. 115, 129–136.

Davidson, E.A., Hart, S.C., Shanks, C.A., Firestone, M.K., 1991. Measuring gross nitrogen mineralization, immobilization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores. Eur. J. Soil Sci. 42, 335–349.

De, J.L.R., Rosado, M., Paneque, M., Miller, A.Z., Knicker, H., 2018. Effects of aging under field conditions on biochar structure and composition: implications for biochar stability in soils. Sci. Total Environ. 613, 969–976.

Dong, X.L., Li, G.T., Lin, Q.M., Zhao, X.R., 2017. Quantity and quality changes of biochar aged for 5 years in soil under field conditions. Catena 159, 136–143.

Fan, C.H., Chen, H., Li, B., Xiong, Z.Q., 2017. Biochar reduces yield-scaled emissions of reactive nitrogen gases from vegetable soils across China. Biogeosciences 14, 2831–2849.

FAO, I, ISRIC, I, 2012. In: FAO (Ed.), JRC: Harmonized World Soil Database (Version 1.2). 1. Rome, Italy and IIASA, Laxenburg, Austria.

Felber, R., Leifeld, J., Horák, J., Neftel, A., 2014. Nitrous oxide emission reduction with greenwaste biochar: comparison of laboratory and field experiments. Eur. J. Soil Sci. 65, 128–138.

Glaser, B., Guggenberger, G., Zech, W., 2001. Black carbon in sustainable soils of the Brazilian Amazon region. In: Swift, R.S., Spark, K.M. (Eds.), Understanding and Managing Organic Matter in Soils, Sediments and Waters. International Humic Substances Society, St. Paul, MN.

Griffis, T.G., Lee, X., Baker, J.M., Russelle, M.P., Zhang, X., Venterea, R., et al., 2013. Reconciling the differences between top-down and bottom-up estimates of nitrous oxide emissions for the US Corn Belt. Glob. Biogeochem. Cycles 27, 746–754.

Hagemann, N., Harter, J., Behrens, S., 2016. Elucidating the impacts of biochar applications on nitrogen cycling microbial communities. Biochar Application. 1, pp. 163–198.

Hagemann, N., Harter, J., Kaldamukova, R., Guzman-Bustamante, I., Ruser, R., Graeff, S., et al., 2017a. Does soil aging affect the N_2O mitigation potential of biochar? A combined microcosm and field study. Glob. Change Biol. Bioenergy 9, 953–964.

Hagemann, N., Joseph, S., Schmidt, H.P., Kammann, C.I., Harter, J., Borch, T., et al., 2017b. Organic coating on biochar explains its nutrient retention and stimulation of soil fertility. Nat. Commun. 8, 1089.

Harter, J., Krause, H.M., Schuettler, S., Ruser, R., Fromme, M., Scholten, T., et al., 2014. Linking N_2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. ISME J. 8, 660–674.

Harter, J., Guzmanbustamante, I., Kuehfuss, S., Ruser, R., Well, R., Spott, O., et al., 2016. Gas entrapment and microbial N_2O reduction reduce N_2O emissions from a biochar-amended sandy clay loam soil. Sci. Rep. 6, 39574.

Harter, J., El-Hadidi, M., Huson, D.H., Kappler, A., Behrens, S., 2017. Soil biochar amendment affects the diversity of nosZ transcripts: implications for N_2O formation. Sci. Rep. 7, 3338.

Hu, W., Zhang, Y., Huang, B., Teng, Y., 2017. Soil environmental quality in greenhouse vegetable production systems in eastern China: current status and management strategies. Chemosphere 170, 183–195.

Huygens, D., Boeckx, P., Templer, P., Paulino, L., Van, C.O., Oyarzun, C., et al., 2008. Mechanisms for retention of bioavailable nitrogen in volcanic rainforest soils. Nat. Geosci. 1, 543–548.

Jones, C.M., Graf, D.R., Bru, D., Philippot, L., Hallin, S., 2013. The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. ISME J. 7, 417–426.

Jones, C.M., Spor, A., Brennan, F.P., Breuil, M., Bru, D., Lemanceau, P., et al., 2014. Recently identified microbial guild mediates soil N_2O sink capacity. Nat. Clim. Chang. 4, 801–805.

Krause, H.M., Hüppi, R., Leifeld, J., El-Hadidi, M., Harter, J., Kappler, A., et al., 2018. Biochar affects community composition of nitrous oxide reducers in a field experiment. Soil Biol. Biochem. 119, 143–151.

Li, B., Bi, Z.C., Xiong, Z.Q., 2017. Dynamic responses of nitrous oxide emission and nitrogen use efficiency to nitrogen and biochar amendment in an intensified vegetable field in southeastern China. Glob. Change Biol. Bioenergy 9, 400–413.

Lin, Y.X., Ding, W.X., Liu, D.Y., He, T.H., Yoo, G., Yuan, J.J., et al., 2017. Wheat straw-derived biochar amendment stimulated N_2O emissions from rice paddy soils by regulating the amoA genes of ammonia-oxidizing bacteria. Soil Biol. Biochem. 113, 89–98.

Liu, Z., Demisie, W., Zhang, M., 2013. Simulated degradation of biochar and its potential environmental implications. Environ. Pollut. 179, 146–152.

Liu, Q., Liu, B., Zhang, Y., Lin, Z., Zhu, T., Sun, R., et al., 2017. Can biochar alleviate soil compaction stress on wheat growth and mitigate soil N_2O emissions? Soil Biol. Biochem. 104, 8–17.

Liu, Q., Zhang, Y.H., Liu, B.J., Amonette, J.E., Lin, Z.B., Liu, G., et al., 2018. How does biochar influence soil N cycle? A meta-analysis. Plant Soil 1, 1–15.

Long, A., Song, B., Fridey, K., Silva, A., 2015. Detection and diversity of copper containing nitrite reductase genes (nirK) in prokaryotic and fungal communities of agricultural soils. FEMS Microbiol. Ecol. 91, 1–9.

Ma, S., Shan, J., Yan, X.Y., 2017. N_2O emissions dominated by fungi in an intensively managed vegetable field converted from wheat–rice rotation. Appl. Soil Ecol. 116, 23–29.

Martin, S.M., Kookana, R.S., Zwieten, L.V., Krull, E., 2012. Marked changes in herbicide sorption–desorption upon ageing of biochars in soil. J. Hazard. Mater. 231, 70–78.
Mia, S., Dijkstra, F.A., Singh, B., 2017a. Aging induced changes in Biochar's functionality and adsorption behavior for phosphate and ammonium. Environ. Sci. Technol. 51, 8359–8367.
Mia, S., Dijkstra, F.A., Singh, B., 2017b. Chapter one - long-term aging of biochar: a molecular understanding with agricultural and environmental implications. In: Sparks, D.L. (Ed.), Advances in Agronomy. 141. Academic Press, pp. 1–51.
Mia, S., Singh, B., Dijkstra, F.A., 2017c. Aged biochar affects gross nitrogen mineralization and recovery: a ^{15}N study in two contrasting soils. Glob. Change Biol. Bioenergy 9, 1196–1206.
Ng, E.L., Patti, A.F., Rose, M.T., Schefe, C.R., Wilkinson, K., Cavagnaro, T.R., 2014. Functional stoichiometry of soil microbial communities after amendment with stabilised organic matter. Soil Biol. Biochem. 76, 170–178.
Orellana, L.H., Rodriguez-R, L.M., Higgins, S., Chee-Sanford, J.C., Sanford, R.A., Ritalahti, K.M., et al., 2014. Detecting nitrous oxide reductase (nosZ) genes in soil metagenomes: method development and implications for the nitrogen cycle. MBio 5 (e01193–14).
Philippot, L., Andert, J., Jones, C.M., Bru, D., Hallin, S., 2011. Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N_2O emissions from soil. Glob. Chang. Biol. 17, 1497–1504.
Prommer, J., Wanek, W., Hofhansl, F., Trojan, D., Offre, P., Urich, T., et al., 2014. Biochar decelerates soil organic nitrogen cycling but stimulates soil nitrification in a temperate arable field trial. PLoS ONE 9, e86388.
Quilliam, R.S., Marsden, K.A., Gertler, C., Rousk, J., DeLuca, T.H., Jones, D.L., 2012. Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. Agric. Ecosyst. Environ. 158, 192–199.
Ravishankara, A.R., Daniel, J.S., Portmann, R.W., 2009. Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. Science 326, 123–125.
Rutting, T., Muller, C., Lieffering, M., Newton, P.N., 2010. Ten years of elevated atmospheric carbon dioxide alters soil nitrogen transformations in a sheep-grazed pasture. Glob. Chang. Biol. 16, 2530–2542.
Saggar, S., Jha, N., Deslippe, J.R., Bolan, N.S., Luo, J., Giltrap, D.L., et al., 2013. Denitrification and N_2O:N_2 production in temperate grasslands: processes, measurements, modelling and mitigating negative impacts. Sci. Total Environ. 465, 173–195.
Sánchez-García, M., Roig, A., Sánchez-Monedero, M.A., Cayuela, M.L., 2014. Biochar increases soil N_2O emissions produced by nitrification-mediated pathways. Front. Environ. Sci. 2, 25.
Shindo, H., Nishio, T., 2005. Immobilization and remineralization of N following addition of wheat straw into soil: determination of gross N transformation rates by ^{15}N-ammonium isotope dilution technique. Soil Biol. Biochem. 37, 425–432.
Spokas, K.A., 2013. Impact of biochar field aging on laboratory greenhouse gas production potentials. GCB Bioenergy 5, 165–176.
Spokas, K.A., Novak, J.M., Venterea, R.T., 2011. Biochar's role as an alternative N-fertilizer: ammonia capture. Plant Soil 350, 35–42.
Toyoda, S., Yano, M., Nishimura, S., Akiyama, H., Hayakawa, A., Koba, K., et al., 2011. Characterization and production and consumption processes of N_2O emitted from temperate agricultural soils determined via isotopomer ratio analysis. Glob. Biogeochem. Cycles 25, 96–101.
Verhoeven, E., Six, J., 2014. Biochar does not mitigate field-scale N_2O emissions in a northern California vineyard: an assessment across two years. Agric. Ecosyst. Environ. 191, 27–38.
Wang, J.Y., Xiong, Z.Q., Yan, X.Y., 2011. Fertilizer-induced emission factors and background emissions of N_2O from vegetable fields in China. Atmos. Environ. 45, 6923–6929.
Wang, C., Lu, H.H., Dong, D., Deng, H., Strong, P.J., Wang, H.L., et al., 2013. Insight into the effects of biochar on manure composting: evidence supporting the relationship between N_2O emission and denitrifying community. Environ. Sci. Technol. 47, 7341–7349.
Wang, R., Gibson, C.D., Berry, T.D., Jiang, Y., Bird, J.A., Filley, T.R., 2017. Photooxidation of pyrogenic organic matter reduces its reactive, labile C pool and the apparent soil oxidative microbial enzyme response. Geoderma 293, 10–18.
Weng, Z., Zwieten, L.V., Singh, B.P., Tavakkoli, E., Joseph, S., Macdonald, L.M., et al., 2017. Biochar built soil carbon over a decade by stabilizing rhizodeposits. Nat. Clim. Chang. 7, 371–376.
Woolf, D., Lehmann, J., Lee, D.R., 2016. Optimal bioenergy power generation for climate change mitigation with or without carbon sequestration. Nat. Commun. 7, 13160.
Xu, H.J., Wang, X.H., Li, H., Yao, H.Y., Su, J.Q., Zhu, Y.G., 2014. Biochar impacts soil microbial community composition and nitrogen cycling in an acidic soil planted with rape. Environ. Sci. Technol. 48, 9391–9399.
Xu, X., Wu, Z., Dong, Y.B., Zhou, Z.Q., Xiong, Z.Q., 2016. Effects of nitrogen and biochar amendment on soil methane concentration profiles and diffusion in a rice-wheat annual rotation system. Sci. Rep. 6, 38688.
Xu, X.Y., Liu, X.R., Li, Y., Ran, Y., Liu, Y.P., Zhang, Q.C., et al., 2017. High temperatures inhibited the growth of soil bacteria and archaea but not that of fungi and altered nitrous oxide production mechanisms from different nitrogen sources in an acidic soil. Soil Biol. Biochem. 107, 168–179.
Zhang, J.B., Müller, C., Cai, Z.C., 2015. Heterotrophic nitrification of organic N and its contribution to nitrous oxide emissions in soils. Soil Biol. Biochem. 84, 199–209.
Zhu, T.B., Zhang, J.B., Cai, Z.C., 2011. The contribution of nitrogen transformation processes to total N_2O emissions from soils used for intensive vegetable cultivation. Plant Soil 343, 313–327.